The Enzymic Dephosphorylation of Casein Fractions in the Presence of H_2O^{18}

In recent publications (1, 2) Anderson and Kelley have reported on the dephosphorylation of casein in H_2O^{18}. These workers showed that 2.3 atoms of oxygen per phosphate molecule were derived from the water of the medium when whole casein was dephosphorylated in the presence of potato acid phosphatase. Previous workers (3, 4) had shown that, when the phosphomonoester bond of small molecules is hydrolyzed in the presence of acid or alkaline phosphatase, one atom of oxygen per phosphate molecule was derived from the water of the medium, indicating P-O fission without exchange. Stein and Koshland (4) found that alkaline phosphatase could catalyze an exchange reaction with inorganic orthophosphate and H_2O^{18} when the enzyme concentration was increased and the incubation time was greatly extended. Anderson and Kelley (1) were unable to demonstrate an exchange reaction when acid phosphatase was incubated in H_2O^{18} with inorganic orthophosphate, even in the presence of dephosphorylated casein. Since these workers found an average of 2.3 atoms of O^{18} incorporated per mole of phosphate formed by enzymic dephosphorylation of casein, this implies an O^{18} exchange at the substrate level, if one assumes the phosphorus is not bound in casein fractions by triester bonds.

TABLE I

Enzymic Dephosphorylation of Casein Fractions in H_2O^{18} [a]

	α-Casein	β-Casein	Whole Casein
Atom per cent excess O^{18} in the medium	1.40	1.40	1.40
Per cent dephosphorylation	82	97	92
Atom per cent excess O^{18} in phosphate oxygen	0.76	1.00	0.89
Atoms O^{18} incorporated per mole of phosphate	2.2	2.9	2.5

[a] Each incubation mixture contained 600 mg. of the casein fraction and 6.0 mg. of calf intestinal mucosa phosphatase in a final volume of 15.0 ml.; 0.15 ml. of 0.1 *M* $MgCl_2$ and 0.3 ml. of 0.2 *M* acetate buffer, pH 5.3, were added. The final pH was adjusted to 6.0–6.3 with NaOH and the reaction was carried out under toluene at 37°C.

During the course of an investigation on the nature of the phosphate linkage in casein fractions, we have enzymically dephosphorylated α-, β-, and whole casein in a medium containing H_2O^{18}. The reaction was carried out at pH 6.0–6.3 using calf intestinal mucosa phosphatase (5) which had been treated with diisopropylfluophosphate as previously described (6), to minimize proteolysis during the long time of incubation (46 hr.). All incubations were carried out at 37°C. under toluene. To insure maximum removal of phosphorus from the casein fractions, the substrate to enzyme ratio used was 50:1, with the enzyme being added in two equal portions at 0 and 22 hrs. The inorganic phosphate formed was isolated as $MgNH_4PO_4$, converted to $K_2 \cdot HPO_4$ and finally $Ba_3(PO_4)_2$, which was analyzed[1] for O^{18} (7). An inspection of the

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results presented in Table I shows on an average between 2 and 3 atoms of O^{18} incorporated for each mole of phosphate hydrolyzed. This is in agreement with the result of Anderson and Kelley (1) for whole casein dephosphorylated with potato acid phosphatase. These results indicate O^{18} incorporation during the over-all reaction and imply the existence of some exchange phenomenon, probably at the level of the casein substrates. Results from this type of experiment alone do not allow any conclusion concerning the nature of the phosphate linkage in casein fractions. However, these results indicate that the phosphate bond is similar in the casein fractions studied. This is in agreement with previous studies (6, 8–10) and in contrast to the conclusions which Perlmann derived from enzyme specificity studies (11).

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